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Neozygites tanajoae sp. nov., a pathogen of the cassava green mite

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Abstract: The fungal pathogen *Neozygites tanajoae* Delalibera Jr., Humber & Hajek sp. nov. (Zygomycetes: Entomophthorales) is being used in Africa as a biological control agent against the introduced cassava green mite (CGM), *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae). This fungus is specific to CGM and has been referred to as *N. floridana* (Weiser & Muma) Remaud. & Keller, a common pathogen of many tetranychid mites. In the present study *N. tanajoae* is investigated at the morphological and molecular levels and physiological attributes of *N. tanajoae* and *N. floridana* are compared. Morphological observations of *N. tanajoae* isolates generally correspond to *N. floridana* and to other mite pathogenic species of *Neozygites*. However, this fungus readily can be distinguished from *N. floridana* based on 18S rDNA sequences, host ranges, nutritional requirements for growth in vitro, tolerances to cold (4 C) and abilities to withstand specific cryopreservation techniques. *N. tanajoae* isolates from Brazil and Africa have identical 18S rDNA sequences but they presented 5.7 and 9.94% pairwise distance from *N. floridana* isolates. *N. tanajoae* proved to differ sufficiently from other mite-pathogenic fungi referred to as *N. floridana* to justify the description of a new species.

Key words: biological control, Entomophthorales, *Mononychellus tanajoa, Neozygites floridana, Tetranychus urticae,* Zygomycetes

INTRODUCTION

The genus of fungal pathogens *Neozygites* includes 15 species, and each species in general has a restricted host range (Keller 1997). Three *Neozygites* species have been described from tetranychid mites: *N. floridana* (Weiser & Muma) Remaud. & Keller, *N. tetranychi* (Weiser) Remaud. & Keller, and *N. adjarica* (Tsintsadze & Vartapetov) Remaud. & Keller. Both *N. tetranychi* and *N. adjarica* are known only from single collections. A close comparison of these three species indicates considerable overlap of taxonomically significant characters such as spore dimensions (Humber et al 1981). *N. adjarica* was described invalidly (with no type designated) and now is considered to be a synonym of *N. floridana* (Keller 1991, Balazy 1993). *N. tetranychi* is known only from the former Czechoslovkia and is related closely to *N. floridana.* Keller (1997) regarded *N. tetranychi* to be distinguished by the slightly larger capilliconidia, the shape of the resting spores and, in particular, their mode of formation. Balazy (1993) said that the roughness of the capilliconidia might be an artifact of preparation techniques. Resting spores in *N. tetranychi* are referred to as azygospores, however this cannot be confirmed from the type material. In contrast to *N. floridana,* resting spores of *N. tetranychi* were observed in cadavers also producing conidia.

Because of uncertainties about the delimitation of species within the genus, the *Neozygites* species associated with spider mites have been referred to either as unidentified species or as *N. floridana. N. floridana* was described by Weiser and Muma (1966) as a pathogen of Texas citrus mite, *Eutetranychus banksi* (McGregor), in Lake Alfred, Florida. This fungus was also pathogenic to the citrus red mite, *Panonychus ulmi* (Kock), and the six-spotted mite, *Eotetranychus sexmaculatus* (Riley), but the rates of infection were much lower than for Texas citrus mite (Selhime and Muma 1966). Since its description, *N. floridana* has been reported in several countries infecting many species of mites in the family Tetranychidae (Kenneth et al 1972, Nemoto et al 1975, Keller 1991, Mietkiewski et al 2000).

An effort to introduce virulent isolates of a species of *Neozygites* from Brazil to control the cassava green mite (CGM), *Mononychellus tanajoae* (Bondar), in Africa resulted in the establishment of a large collection of isolates of *Neozygites* spp. from mites. The CGM pathogen initially was referred to as *Neozygites* sp. (Delalibera Jr. et al 1992) and later as *N. floridana* (Oduor et al 1995, Keller 1997, Elliot et al 2000). Since this pathogen was first found in Brazil in 1988,

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considerable qualitative and quantitative data have been accumulated on epizootiological, morphological and physiological aspects of strains from Brazil, Colombia and Benin. As more information about CGM-pathogenic *Neozygites* accumulated, consistent differences with *N. floridana* became evident. This study was undertaken to compare the CGM-pathogenic *Neozygites* with *N. floridana*.

Traditional classifications of *Neozygites* species have been based on morphological characters, mainly spore size and host. In this study, the morphology of four isolates of the CGM pathogen from three states in Brazil is compared with published data from other mite-pathogenic strains of *Neozygites*. Comparisons of sequences of the small subunit of ribosomal DNA (18S rDNA) for CGM-pathogenic isolates from Brazil and from Benin and isolates pathogenic to the two-spotted spider mite, *Tetranychus urticae* Koch, from Colombia and the United States, also are presented. The molecular and morphological information then is correlated with other physiological characteristics presented by Delalibera Jr. (2002). All observations together indicated the appropriateness of describing the Brazilian and African CGM pathogen as a new species, *Neozygites tanajoae* Delalibera Jr., Humber & Hajek sp. nov.

MATERIALS AND METHODS

Morphological comparison.—Morphological measurements were taken on *N. tanajoae* isolates from Gravatá, Pernambuco (BIN 16), São Miguel das Matas, Bahia (BIN 9), Floriano, Piauí (BIN 21), and Araripina, Pernambuco (BIN 22) from Brazil (TABLE I).

Neozygites isolates were produced individually in vivo in the laboratory for morphological observations. A single mummified mite from each *N. tanajoae* isolate that had been maintained 3 y at −10 C was placed on a leaf disk overnight to sporulate and infect CGM. The spores were used to infect CGM using the method described by Delalibera Jr. (1996) with mites from a laboratory colony of CGM from Cruz das Almas, Bahia, Brazil. The resulting mummified mites were allowed to sporulate 12–24 h on microscope slides. Discharged primary conidia and capilliconidia were mounted in lactophenol-aceto-orcein. Dark cadavers formed during in vivo production of isolates were mounted in lactophenol-cotton blue for observation of resting spores. Measurements were made on 30 fungal structures produced from each mite; this is referred to as one series.

Comparison of 18S rDNA sequences.—The 18S rDNA of two isolates of CGM-pathogenic *Neozygites* from Brazil (BIN 16, BIN 10), one isolate from Benin (BIN 35) as well as *N. floridana* from Colombia (ARSEF 5376) and North America (ARSEF 662) was sequenced partially. In vitro isolates BIN 16, BIN 10 and BIN 35 were deposited at the National Center for Genetic Resources and Biotechnology Research (CENARGEN) of the Brazilian Organization for Agricultur-

TABLE I. Identification and origin of collection of *Neozygites* spp. isolates

In vivo collection[a]	Location	Latitude[b]	Longitude[b]	Host	In vitro collection[c]	GenBank accession No.
BIN08	Cruz das Almas, Bahia, Brazil	12°40′19″ S	39°06′23″ W	*M. tanajoa*	CG 872	
BIN09	S. M. das Matas, Bahia, Brazil	13°01′00″ S	39°25′00″ W	*M. tanajoa*		
BIN10	Tiangua, Ceará, Brazil	04°06′33″ S	40°53′15″ W	*M. tanajoa*	CG 867	AY233981
BIN16	Gravatá, Pernambuco, Brazil	8°11′01″ S	35°29′26″ W	*M. tanajoa*	CG 871	AY233982
BIN21	Floriano, Piauí, Brazil	6°38′28″ S	42°52′14″ W	*M. tanajoa*		
BIN22	Araripina, Pernambuco, Brazil	7°30′03″ S	40°27′42″ W	*M. tanajoa*		
BIN35	Cotonou, Benin	6°25′ N	2°18′ E	*M. tanajoa*	CG 873	AY233983
	North Carolina, USA			*T. urticae* and other tetranychid mites	ARSEF662	AF296759 AY233985
	Palmira, VA, Colombia	3°32′ N	76°16′ W	*T. urticae* and *M. tanajoa*	ARSEF5376	AY233984

[a] BIN = Identification of the collection of *Neozygites* isolates in vivo, EMBRAPA—Cassava and Tropical Fruits, Cruz das Almas, Bahia, Brazil.

[b] Locations with detailed latitude and longitude were determined with Global Positioning System and others were extrapolated using the distance of the collection site to the nearest city of a known latitude and longitude.

[c] CG = EMBRAPA—National Center for Genetic Resources and Biotechnology Research, Brasilia, Brazil and ARSEF = Agricultural Research Service Collection of Entomopathogenic Fungal Cultures.

al Research (EMBRAPA) under numbers CG871, CG867 and CG873, respectively.

DNA was extracted from fresh hyphal bodies produced in vitro in IPL-41 medium supplemented with 5% fetal bovine serum +0.3% lactalbumin hydrolysate and +0.3% yeastolate (NT-1 medium) (Delalibera Jr. 2002). Hyphal bodies were collected by centrifugation for 10 min at 1300 rpm. The DNA extraction was carried out using DNeasy tissue kits (Qiagen Inc.). SSU rDNA was amplified using the fungal universal primers nu-SSU-0021-5′ (5′-CTGGTTGATTCTGCCAGT-3′; Gargas and DePriest 1996) and nu-SSU-1780-3′ (5′-AATGATCCTTCCGCAGGT-3′; DePriest 1993).

PCR reactions were conducted with an initial denaturation for 3 min at 94 C, followed by 35 cycles with denaturation for 1 min at 94 C, annealing for 1 min at 50 C, extension 2.5 min at 72 C and final extension for 10 min at 72 C. The PCR reactions were carried out using a Hybaid OmniGene thermal cycler in 50 μL volumes using Taq PCR Core kits (Qiagen Inc.), following the company's recommendations: 200 μM of each dNTP, 15 mM $MgCl_2$, 2.5 units Taq DNA polymerase, 1× Taq Buffer, 0.3 μM of each primer and 10–100 ng DNA template. PCR-amplified products were gel-purified in 1.5% agarose gel in 1× TAE buffer, and the products were visualized with ethidium bromide. PCR products were sequenced on both strands using PCR primers and the internal primers 5′-GATTAGATACCGTTGTAGTCTCA-3′, 5′-TGGATAGCAAGGCATAGCGAG-3′, 5′-TGAGCCTTTCGCGGTGTTG-3′ and 5′-TGAGACTACAACGGTATCTAATC-3′. Sequencing was conducted at the Cornell University DNA sequencing facility. Sequences were aligned with other 18S rRNA sequence of *N. floridana* (GenBank accession No. AF296758) using the Clustal X (1.81) program (Thompson et al 1997). This *N. floridana* sequence is from an isolate collected in Switzerland infecting *T. urticae* (F. Freimoser pers comm). Alignments were refined further using the Bioedit sequence alignment editor program version 5.0.9 (Hall 1999). Pairwise distances among isolates were calculated using Kimura's two-parameter method available in the DNADIST (PHYLIP 3.6 program) on 1397 alignment positions.

RESULTS

Morphological comparison.—Range of means, and minimum and maximum values are presented (TABLE II). Data collected from the literature on these other *Neozygites* species pathogenic to tetranychid mites are included for comparison: (i) the original description of *N. floridana* by Weiser and Muma (1966) pathogenic to *E. banksi*; (ii) *N. floridana* from *T. urticae* from Switzerland and North Carolina and from CGM from Benin (Keller 1997); (iii) *Neozygites* sp. from *M. tanajoa* (= *M. progressivus*) from Venezuela (Agudelo-Silva 1986); and (iv) *Neozygites* sp. pathogenic to *T. evansi* from Brazil (Humber et al 1981). The magnitude of maximum to minimum values demonstrates the tremendous variation in *Neozygites* structures. Measurements of fungal structures of all isolates of *N. tanajoae* examined in this study overlapped with all *N. floridana* compared (TABLE II). No significant variations were observed in the taxonomic characters investigated (size and shape of primary conidia, capilliconidia, capillary tubes and resting spores).

Comparison of 18S rDNA sequences.—Pairwise comparisons on 1397 aligned positions of 18S rRNA genes showed that the two Brazilian isolates of *N. tanajoae* and one isolate from Benin are 100% similar. The *N. floridana* isolates from Colombia and the United States have identical 18S rDNA sequences. However, *N. tanajoae* sequences diverged 5.7% from *N. floridana* from Colombia and the United States and 9.94% from sequence AF296758 from Switzerland. In fact, the *N. floridana* from Colombia and the United States are more similar to *N. tanajoae* isolates than they are to *N. floridana* AF296758 (8.24% divergence). Sequences of *N. tanajoae* and *N. floridana* were deposited in the GenBank database (National Center for Biotechnology Information, Bethesda, Maryland) under accession numbers AY233981–AY233985 (TABLE I).

Comparison of host range, in vitro growth and tolerance to cryopreservation.—*N. tanajoae* is a pathogen specific to CGM and does not infect mite species susceptible to *N. floridana,* such as *Tetranychus bastosi* Tuttle, Baker & Sales (Moraes and Delalibera Jr. 1992), *T. urticae* and *Oligonychus gossypii* (Zacher) (Delalibera Jr. 2002). Although the degree of host specificity of *N. floridana* is not known, some strains are known to have a wider host range than *N. tanajoae* (Delalibera Jr. 2002, Butt and Humber 1989, Selhime and Muma 1966).

N. tanajoae presents remarkable differences in nutritional requirements and ability to withstand cryopreservation and the stress of cold (4 C) compared to *N. floridana* (TABLE III) (Delalibera Jr. 2002). *N. tanajoae* is a particularly fastidious species and grows only in a restricted number of media, while isolates referred to *N. floridana* grow faster and in a broader range of media, including serum-free media. Hyphal bodies of *N. tanajoae* isolates in vitro are shorter than hyphal bodies of the *N. floridana* isolates. In vitro cultures of two *N. floridana* isolates remained viable at 4 C up to 47 d, while cultures of *N. tanajoae* could not be subcultured after maintenance at this temperature for as little as 4 d. *N. tanajoae* has a lower tolerance to freezing. Successful cryopreservation methods for *N. tanajoae* isolates are both unusual in comparison to those for many fungi and not suitable for *N. floridana* isolates.

TABLE II. Synopsis of structures (μm) of *Neozygites tanajoae* isolates pathogenic to *Mononychellus tanajoa* (CGM), and described *N. floridana* pathogenic to other tetranychid mites. Adapted from Keller (1997)

Fungal structure	Isolate[a]/host	N[f]	Length		Width	
			Range of means[g]	Extreme min–max	Mean[g]	Extreme min–max
Primary conidia	*floridana* type[b]	5	15	13–18	12	11–13
	BIN 16/CGM	5	14.2–15.6	12–17	12.2–14	10–16
	BIN 9/CGM	3	13.7–14.5	12–16	12.2–12.8	11–15
	BIN 22/CGM	3	14.2–15.5	12–17	12–13.6	10–15
	BIN 21/CGM	2	15–16.4	13–18	13.7–14.9	12–17
	BIN 8/CGM[c]	6	13.7–15.3	12–18	11.6–12.4	10–16
	SK/TSSM[c]	5	13.9–14.8	12–18	11.4–12.7	10–16
	JSY/TSSM[c]	3	13.8–14.1	12–16	11.3–11.8	10–13
	PAS/CGM[d]		(16)15–17		(14)12–15	
	RAH/TE[e]		(14.9)12.4–17.7		(12)9.4–14.2	
Capilliconidia	*floridana* type[b]			15–20		10–12
	BIN 16/CGM	5	18–19.9	15–23	10.1–10.7	8–14
	BIN 9/CGM	3	17.2–17.5	15–20	9–9.8	8–13
	BIN 22/CGM	3	18.3–19.1	15–22	9.7–10.8	9–13
	BIN 21/CGM	3	16–18.2	12–20	9.6–11.9	8–13
	BIN 8/CGM[c]	5	15.2–18.5	13–22	9.2–10.8	8–12
	SK/TSSM[c]	5	17.2–18.9	15–22	8.3–9.3	7–11
	DRS/TSSM[c]	1	18.2	17–19	8.7	7–11
	PAS/CGM[d]		(19)12–21		(10)8–12	
	RAH/TE[e]		(18.2)15.3–20.1		(9.6)7.1–13	
Capillary conidiophore	*floridana* type[b]			50–60		
	BIN 16/CGM	4	46.1–53.6	32–75		
	BIN 9/CGM	3	46.4–49.2	32–63		
	BIN 22/CGM	3	53.9–56.8	38–73		
	BIN 21/CGM	2	52.4–61.9	43–75		
	BIN 8/CGM[c]	4	45–50	28–69		
	SK/TSSM[c]	2	42.7–48.8	25–65		
	DRS/TSSM[c]	1	58.7	42–70		
Resting spore diameter	*floridana* type[b]			22–26		20–24
	BIN 16/CGM	4	17.8–23.1	16–26	18.3–23.1	16–25
	SK/TSSM[c]	8	19.3–23.1	16–25	14.6–16.6	13–18
	DRS/TSSM[c]	3	19.6–22.1	18–27	14.6–16.6	13–18

[a] BIN = *Neozygites* isolates from EMBRAPA-Cassava and Tropical Fruits fungal collection, Cruz das Almas, Bahia, Brazil. SK = S Keller, DRS = DR Smitley, PAS = P Agudelo-Silva, RAH = RA Humber, JSY = JS Yaninek, CGM = cassava green mite, TSSM = twospotted spider mite, TE = *Tetranychus evansi*.

[b] Weiser & Muma (1966).

[c] Keller (1997).

[d] Agudelo-Silva (1986).

[e] Humber et al. (1981).

[f] Number of series of 30–50 measurements.

[g] Data in parentheses indicate averages of means.

TAXONOMY

Neozygites tanajoae.—Delalibera Jr., Humber & Hajek sp. nov FIGS. 1–9

Corpora hyphoidea bacilliformes, 24–38 × 8.2–10.1 μm in vitro, nucleis saepe 3–5 (2–10 in hospite, 2–14 in vitro). Conidiophora simplicia, conidia apicali unico, ex corporibus hyphoidibus singulariter emergentia. Conidia primaria globosa ovoidea, 13.7–16.4 × 11.6–14.9 μm, papilla brevi rotundata truncatave, per eversione papillae expulsis; conidia secundaria vel conidia primaria simulantia in conidiophoro brevi crasso vel conidiophoro capilliformi, 45–61.9 × 1–2 μm, geniculo infraapicale formantia; capilliconidiis amygdaliformibus, 15.2–19.9 × 9.2–11.9 μm, fuscis guttula mucosa apicali plerumque singulariter vel rare 2–3 parvioribus per conidio ex conidio primario formantia; conidiis tertiariis quarternariis conidia secundaria aur parviora seriatim simulantia. Sporae perdurantae subgloboae, 17.8–

TABLE III. Characters that discriminate between *Neozygites tanajoae* and *N. floridana* (from Delalibera Jr. 2002)

Character	*N. tanajoae*	*N. floridana*
Pathogenicity to *Tetranychus uticae*	−	+
Number of suitable culture media that afford more than 2×10^6 hyphal bodies/mL (out of 9 media tested)	3	≥7
Cryopreservation in 5% glycerol	−	+
Cryopreservation in 1% trehalose + 2% DMSO	+	−
Length of hyphal bodies in vitro (μm)[1]	24–38	42–44
Viability of in vitro culture at 4 C (days)	4	47
Genetic distance (%) based on 18S rDNA	5.7–9.94%	

[1] Measurements taken 3 d after inoculation in fresh IPL-41 supplemented medium.

23.1 μm diam, binucleatae, brunnea, superficiebus exosporarum asperis; seu zygosporae seu zaygosporae originibus incertis; in acaris conidiiferentia desunt. Cystidia ignotae. Rhizoidea a nobis inobservata aut Kellero (1997) in speciminibus Mononychello tanajoa basiliensibus sporis perdurantis includentibus visis.

Hyphal bodies rod-shaped, 24–38 × 8.2–10.1 μm (in vitro; not measured from hosts), mostly 3–5 nucleate (but 2–10 in vivo and 2–14 in vitro). Conidiophores unbranched, forming singly on hyphal bodies and forming a single apical primary conidium. Primary conidia globose or ovoid, 13.7–16.4 × 11.6–14.9 μm with a short, rounded or truncated papilla, forcibly discharged by papillar eversion. Secondary conidia either similar to primary conidia, formed on short, thick conidiophore and forcibly discharged or capilliconidia passively dispersed from atop a capillary conidiophore; capilliconidia almond-shaped, 15.2–19.9 × 9.2–11.9 μm, pale brown, with a droplike mucoid haptor at the apex, usually produced singly on any primary conidium but rarely as many as three small capilliconidia produced on a primary conidium; capillary conidiophores 45–61.9 × 1–2 μm with S-shaped or geniculate bend at the apex. Tertiary and quaternary conidia are similar in shape but progressively smaller in size than conidia from which they arise, sometimes formed after more than 24 h at high relative humidity. Resting spores subglobose, 17.8–23.1 μm diam, binucleate, dark brown, with a roughened surface; mode of formation as zygospores or azygospores remains unconfirmed; never observed to be formed in mites producing conidia. Cystidia unknown. Rhizoids not observed in isolates investigated in this study although Keller (1997) observed rhizoids on Brazilian cadavers of cassava green mite containing resting spores.

Etymology.—*Tanajoae* refers to the specific name of the host, *M. tanajoa,* described by Bondar in 1938 based on specimens collected in Bahia, the same state where *N. tanajoae* first was found in Brazil and from which the holotype was collected. ''Tanajoa'' is the local name farmers of northeastern Brazil attribute to the damage *M. tanajoa* causes on cassava.

Holotype.—Brazil. Cruz das Almas, Bahia: A slide containing three sporulated cadavers of *M. tanajoa* and microcentrifuge vial containing 25 mycotized cadavers and mummies; all cadavers generated from isolate (BIN 8) of *N. tanajoae* collected by Italo Delalibera Jr. on 5 Nov 1993. and maintained in vivo. Deposited in CUP (Cornell University, Plant Pathology Herbarium) as CUP 65749.

Paratypes.—ARSEF slides and collection of I. Delalibera Jr., slides with sporulated cadavers and mummified adult females of *Mononychellus tanajoa.*

Type host.—*Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), adult females.

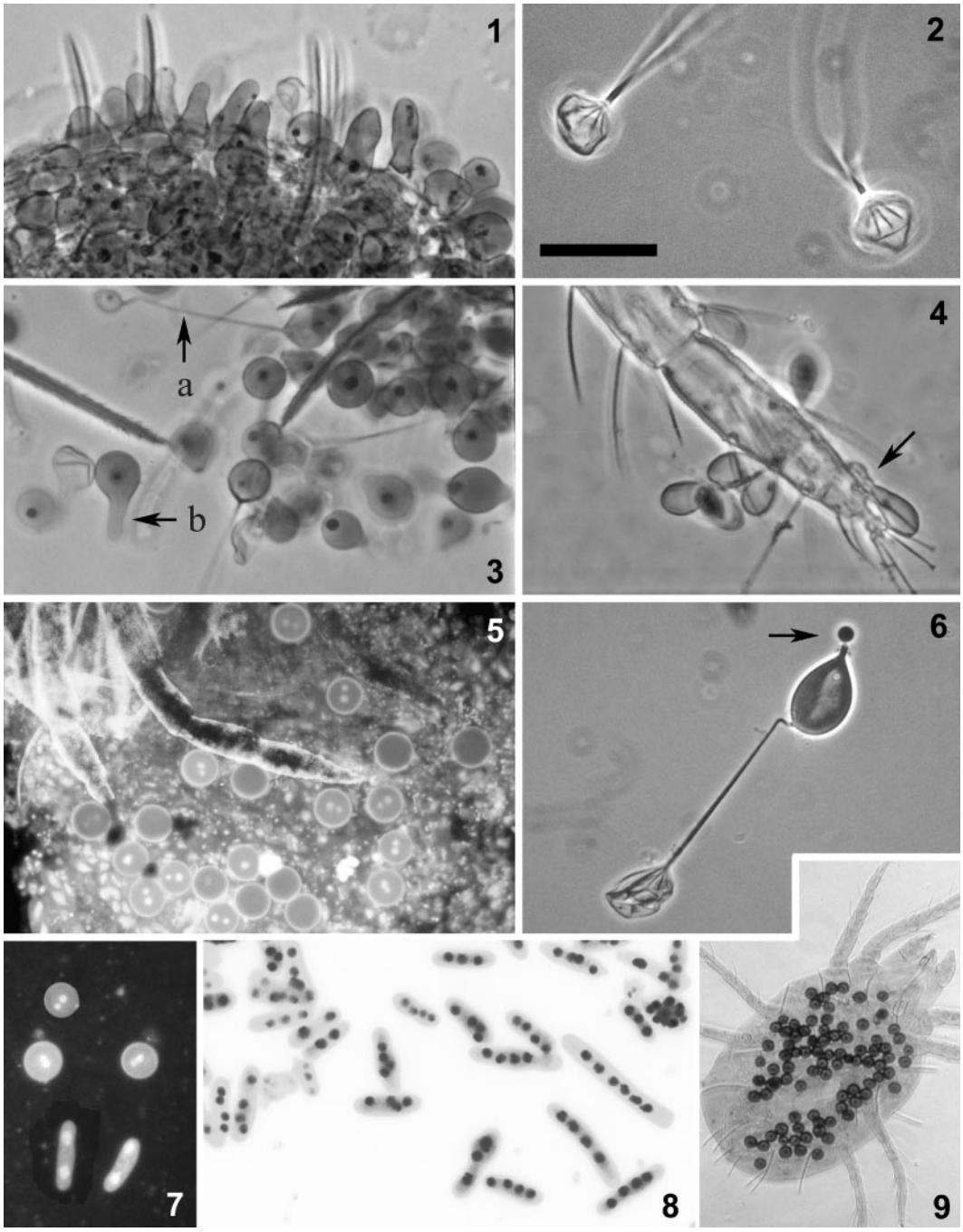
Type locality.—Cruz das Almas, Bahia, Brazil.

Culture ex type.—Microcentrifuge vial containing 10 mycotized cadavers and mummies of *N. tanajoae* maintained in vivo; all cadavers generated from Gravatá, Pernambuco (BIN 16); São Miguel das Matas, Bahia (BIN 9); and Floriano, Piauí (BIN 21) from Brazil. Deposited in CUP (Cornell University, Plant Pathology Herbarium) as CUP 65751, CUP 65750 and CUP 65752, respectively. Isolates are identified also by collection of I. Delalibera Jr., slides with sporulated cadavers and mummified adult females of *Mononychellus tanajoa.*

Other cultures examined.—Nineteen isolates from nine Brazilian states and four isolates from Benin used for investigations of host range, nutritional requirements cryopreservation and cold tolerance are listed by Delalibera Jr. (2002).

DISCUSSION

In the latest review of the *Neozygites,* Keller (1997) examined several collections of *Neozygites* associated with tetranychid mites, including two isolates collected from CGM in Brazil and one from CGM in Benin. Materials from these three locations as well as the other tetranychid-infecting fungi were assigned to *N. floridana.* The morphometric analyses of more isolates of the CGM pathogen conducted during this study cannot help to distinguish the CGM pathogen



FIGS. 1–9. *Neozygites tanajoae*. 1. Conidiophores; bar = 30 μm. 2. Primary conidia remaining after germination; bar = 20 μm. 3. Primary conidia germination (a) capillary conidiophore (b) germ tube; bar = 30 μm. 4. Capilliconidium germinating on *Mononychellus tanajoa* leg, arrow indicates the germ tube; bar = 30 μm. 5. Two-nucleate resting spores; bar = 60 μm. 6. Capilliconidium on bent apex of capillary conidiophore produced on primary conidium, arrow indicates haptor with mucoid apical droplet; bar = 27 μm. 7. Two-nucleate hyphal bodies and resting spores in vivo; bar = 60 μm. 8. Hyphal bodies in vitro; bar = 60 μm (inverted image). 9. Resting spores inside *M. tanajoa*. FIGS. 1, 3, 4 mounted in lactophenol cotton blue and FIGS. 5, 7, 8 stained with propidium iodide.

from other isolates of *Neozygites* pathogenic to tetranychid mites. The morphology of *N. tanajoae* isolates generally correspond to all fungi attributed to *N. floridana*. Due to the structural simplicity of these fungi and difficulties in investigating more unusual structures, such as resting spores and rhizoids, the amount of morphological data useful for classification is limited. Humber et al (1981) suggested that the full

range of morphological variation is not understood for fungi identified at least provisionally as *N. floridana*. Although standard morphological criteria are not useful for specific identification, the CGM pathogen displays various physiological, pathobiological and molecular characteristics markedly different from *N. floridana* but consistent among all isolates being named as *N. tanajoae* (TABLE III).

We have demonstrated that supplementing classical taxonomic criteria with physiological and molecular data is both useful and practicable for the differentiation of *N. tanajoae* from the morphologically similar *N. floridana*. Studies of this magnitude have yet to be done with other strains attributed to *N. floridana* to assess the degree of intraspecific and interspecific variation. *N. floridana* is distributed globally and pathogenic to several species within the mite family Tetranychidae. The pathobiology of *N. floridana* must be investigated more completely to understand the degree of host specificity of this group of pathogens. *N. floridana* has been associated with CGM, *T. urticae* and *Oligonychus gossypii* (Acari: Tetranychidae) on cassava in Colombia and Africa (Alvarez Afanador 1990, Yaninek et al 1996). However, Delalibera Jr. (2002) demonstrated that the fungus associated with CGM is not the same fungus that infects the other two species. Ribosomal DNA sequences from another fungus identified as *N. floridana* (GenBank accession No. AF296758) also pathogenic to *T. urticae* (F. Freimoser pers comm) demonstrated a large genetic distance with sequences from the two *N. floridana* isolates presented in this study. Although morphologically similar, these two groups are distinct genetically suggesting the occurrence of further distinct species within *N. floridana*. Occurrences of species complexes are common in the Entomophthorales (Hajek et al 2003). *N. floridana* should be treated as a partially resolved species complex, from which *N. tanajoae* is the first recognized segregate, until comprehensive studies can be performed to further elucidate the taxonomic status of this group.

This study has shown that ribosomal DNA sequences are good tools for phylogenetic analyses of mite-pathogenic species of *Neozygites* because of the relatively small number of useful morphologic characters. The SSU rDNA and other genes of more *N. floridana* isolates must be sequenced to increase our knowledge about the molecular phylogeny of this group of pathogens.

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